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MOLECULAR DATA CONFIRM THE PHYLOGENETIC PLACEMENT OF THE ENIGMATIC *HESPEROCALLIS* (HESPEROCALLIDACEAE) WITH *AGAVE*

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Abstract

Hesperocallis is a monotypic genus endemic to western North America, currently classified in Hesperocallidaceae (*sensu* Angiosperm Phylogeny Group 1998, 2003) but typically placed in Liliaceae (*sensu* Cronquist 1988) in floristic treatments. On the basis of DNA sequence data, the phylogenetic placement of *Hesperocallis* is demonstrated to be with Agavaceae, rather than with Alliaceae, Hemerocallidaceae, or Liliaceae as thought by earlier authors based on morphology. Based on these results, we recommend sinking Hesperocallidaceae in Agavaceae within Asparagales.

Resumen

Hesperocallis es un género monotípico endémico del oeste de América del Norte. Actualmente, *Hesperocallis* está clasificado dentro de Hesperocallidaceae (Angiosperm Phylogeny Group 1998, 2003); sin embargo, previas clasificaciones basadas en morfología lo han situado al interior de Liliaceae (*sensu* Cronquist 1988). Con base en secuencias de ADN, *Hesperocallis* esta filogenéticamente emparentado con la familia Agavaceae y no con Alliaceae, Hemerocallidaceae o Liliaceae como fue sugerido por previos autores. Como resultado, recomendamos considerar a Hesperocallidaceae como parte de Agavaceae dentro de las Asparagales.

Key Words: *Agave,* Agavaceae, Asparagales, *Hesperocallis,* Liliaceae, molecular systematics, phylogeny.

Asa Gray (1867) described *Hesperocallis* as a monotypic genus of Liliaceae. The sole species, *H. undulata,* occurs on sandy flats and mesas of creosote scrub in the Mojave Desert and Sonoran Desert (USA: Arizona, California, and Nevada; Mexico: central Baja California and Sonora; Wiggins 1980; McNeal 1993; Utech 2002). *Hesperocallis* is a perennial herb, with mostly basal linear leaves and a scapose inflorescence arising from a tunicate bulb. The leaves are distinctively keeled, strongly undulate, and blue-green with white margins. Commonly known as the desert glory lily, it has large white flowers that make it not only one of the most attractive desert species, but also a plant that has attracted horticultural use (Utech 2002). Native Americans used the bulbs for food (Moerman 1986), and the early Spanish colonists called the bulbs *ajo,* due to their garlic smell. However, Gray's description of *Hesperocallis* did not note the alliaceous scent, perhaps because he described the plant from a dried specimen, collected by J.G. Cooper, a botanist with the U.S.-Mexican Boundary Survey from 1860–1861. In fact, Gray (1867) thought that *Hesperocallis* was related to *Hemerocallis,* and the generic name was intended to suggest that affinity, "along with the far western, instead of eastern habitat" (*Hesperocallis* is Greek for "western beauty"). Rowntree (1941) provided an early natural history essay of *Hesperocallis,* Maddox and Carlquist (1985) studied seed dispersal, and recent studies and floras contain descriptions and plates of *Hesperocallis* (e.g., McNeal 1993; Utech 2002). North American floras typically follow Cronquist's (1988) taxonomic scheme and continue to place *Hesperocallis* in Liliaceae; however,

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recent authors have noted the artificiality of Cronquist's Liliaceae (Duvall et al. 1993; McNeal 1993; Chase et al. 1995; Reveal and Pires 2002; Utech 2002).

Hesperocallis, like many monotypic genera of petaloid monocots, has puzzled plant systematists for decades. Hutchinson (1934, 1959) placed *Hesperocallis* and *Hosta* in his Hemerocallidaceae (Liliales), separate from his order Agavales. Cave (1948, 1970) found that *Hesperocallis* was karyologically and embryologically similar to *Hosta* (Hostaceae) and some genera of Agavaceae. Even though their base chromosome numbers are different ($\bar{x}$ = 24 in *Hesperocallis* and $\bar{x}$ = 30 in *Hosta* and Agavaceae), they share a strongly bimodal karyotype. Although Cave (1948) suggested the removal of *Hesperocallis* and *Hosta* from Hemerocallidaceae, Hutchinson (1959) did not alter his 1934 classification of *Hesperocallis* and maintained it in Hemerocallidaceae. Dahlgren, Clifford and Yeo (1985, pg. 187) placed the North American *Hesperocallis* and *Leucocrinum* in Funkiaceae (=Hostaceae) but were uncertain about the relationship of this family to the other 29 families of their Asparagales. Later workers (Alvarez and Köhler 1987) found that the pollen grains of *Hesperocallis, Hosta,* and *Leucocrinum* have similar unibaculate muri that differed from the pollen morphology of genera traditionally placed in Agavaceae.

In contrast, Traub (1953, 1982) did not believe there was a close relationship between *Hesperocallis* and *Hosta* and placed *Hosta* in tribe *Hosteae* of Agavaceae (Agavales *sensu* Hutchinson 1934, 1959; Traub 1953, 1972b). For *Hesperocallis,* Traub emphasized its alliaceous scent and hypothesized a relationship with Alliaceae. Traub (1968) initially placed *Hesperocallis* in its own tribe and then later in its own family Hesperocallaceae in his order Alliales (Traub 1972a, 1982). Traub (1982) referred to *Hesperocallis* and *Milula spicata* as "living fossils" and postulated that they represented ancestral lineages similar in form to the extinct ancestors of Alliales.

Hesperocallis is currently treated as the sole representative of the segregate family Hesperocallidaceae within Asparagales (Angiosperm Phylogeny Group, APG 1998; APG II 2003). APG (1998) left Hesperocallidaceae unplaced within the Asparagales because it had not been included in any molecular phylogenetic analyses. Fay et al. (2000) produced a molecular analysis of Asparagales based on *rbcL, atpB,* and *trnL-F* plastid DNA sequences, which clarified relationships within Asparagales and was the basis of the most recent classification of the order (APG II 2003). However, Fay et al. (2000) did not sample *Hesperocallis* and identified *Hesperocallis* as a critical taxon to be included in future studies. Fay et al. (2000) suggested that *Hesperocallis* might have affinities with Agavaceae, a reasonable hypothesis given that *Hosta* had been found to be related to *Agave* in previous molecular studies (Bogler and Simpson 1995, 1996; Chase et al. 1995). To determine whether *Hesperocallis* has affinities with Alliaceae, Agavaceae, or Hemerocallidaceae, we present the first molecular phylogenetic analysis of *Hesperocallis* using the combined DNA matrix for Asparagales of Fay et al. (2000), to which we have added new data for *Hesperocallis.*

Materials and Methods

Material of *Hesperocallis* was collected into silica gel using the method of Chase and Hills (1991). Two accessions of *Hesperocallis* were used in this study that were collected at different localities from within the Anza-Borrego Desert State Park, San Diego County, in southern California. One voucher is deposited at SD (*Rebman 7176,* SD 148685) and the other at JEPS (*Cranfill & Schmid, s.n.*). DNA extraction and sequencing were carried out using standard techniques (Fay et al. 2000). Newly determined *rbcL* (accession number AY561251), *atpB* (accession number AY561252), and *trnL-F* (accession number AY561253) sequences for *Hesperocallis* have been deposited in the GenBank database. Insertions/deletions (indels) were introduced to the *Hesperocallis trnL-F* sequence to align it to the matrix.

Using the parsimony algorithm of the software package PAUP* for Macintosh (version 4.0b10; Swofford 2002), a tree search was conducted on the combined *rbcL/atpB/trnL-F* matrix under the Fitch (equal weights) criterion (Fitch 1971) with 1000 random sequence additions and tree-bisection-reconnection (TBR) branch swapping, but permitting only five trees to be held at each step. All shortest trees collected in the 1000 replicates were swapped on to completion with no tree limit. Successive approximation weighting was carried out according to the rescaled retention index (RI), using the maximum value (best fit) and a base weight of 1.0. A new heuristic search was performed with 1000 random sequence additions, TBR swapping and holding five trees per step; the reweighting/heuristic search combination was repeated until the number of trees found and tree length became consistent. Internal support was evaluated with equal weights using 1000 replicates of the bootstrap (BS; Felsenstein 1985), with simple sequence addition and TBR swapping, but permitting only five trees to be held at each step.

Results and Discussion

The aligned data matrices were unchanged from the original Fay et al. (2000) matrix except for the addition of the three sequences for *Hesperocallis.* The total aligned matrix was 4857 characters (*rbcL* accounted for 1428 base pairs, bp, *atpB* for 1518 bp, and the *trnL-F* region for 1911 bp, respectively). A total of 1306 base positions were excluded

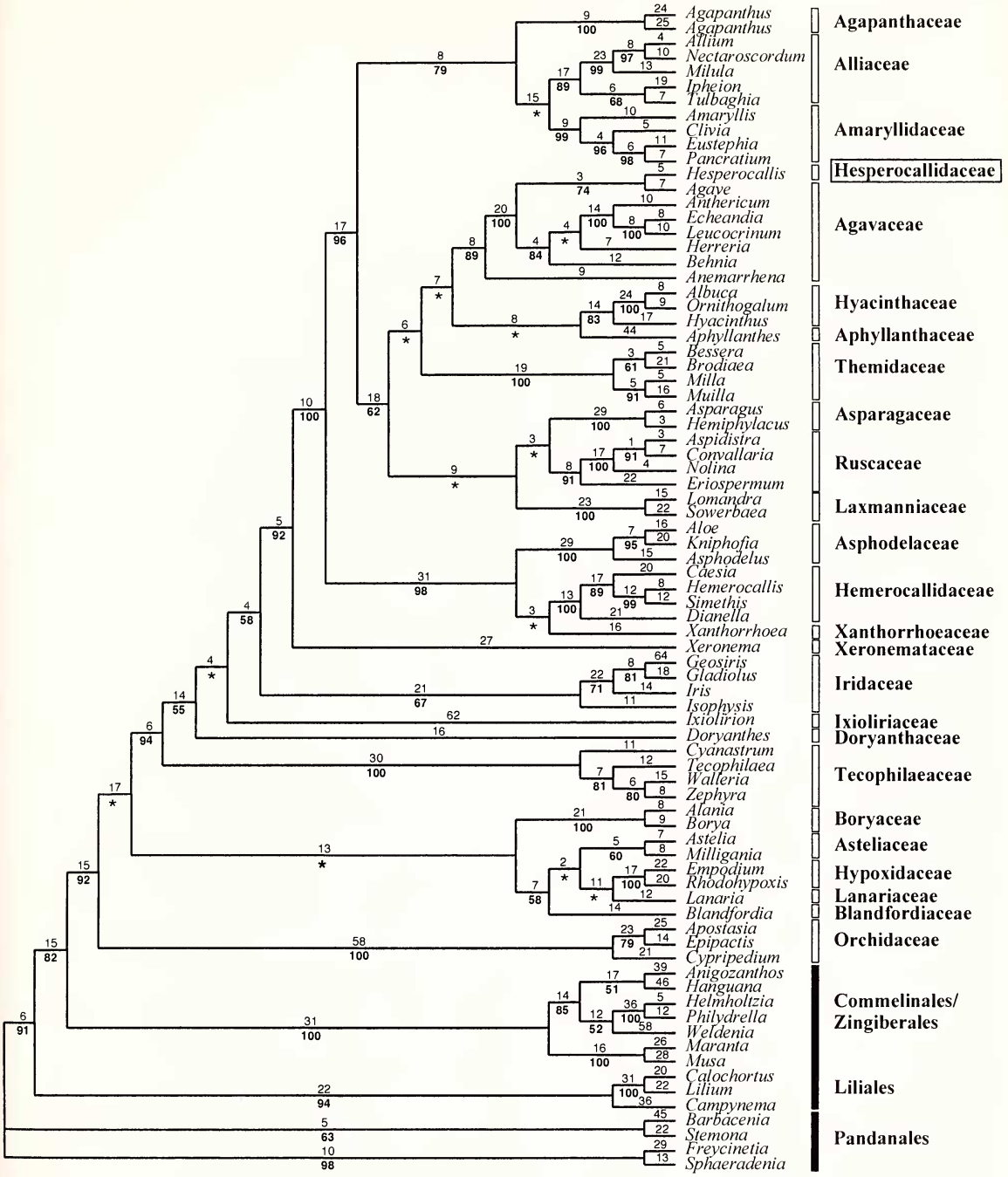

FIG. 1. Single most parsimonious tree of Asparagales (obtained after successive weighting) based on *rbcL, atpB,* and *trnL-F* plastid DNA sequences. Branch lengths are shown above the branches and bootstrap percentages (produced with equal weighting) are shown below the branches (asterisks indicate <50% BS). Dark bars to right of the tree indicate outgroup orders. Open bars indicate the 25 narrowly bracketed families of Asparagales (APG II 2003). Note that Hesperocallidaceae are in a clade containing Agavaceae and not with Alliaceae or Hemerocallidaceae.

either at the beginning or end of sequences or where alignment of the *trnL-F* sequences proved too difficult to align clearly (Fay et al. 2000). Of the 3551 included characters, 1479 (42%) were variable and 958 (27%) were potentially parsimony informative.

The combined Fitch analysis produced 18 equally most-parsimonious trees, tree length (TL) =

4721, consistency index (CI) = 0.43 and retention index (RI) = 0.55. One of these trees was selected as optimal under the weighting criterion, 2229 weighted steps, CI = 0.55 and RI = 0.69 (Fig. 1, with its Fitch branch lengths shown above the branches and bootstrap percentages, BS, below). As expected, the overall topology of the tree is similar to that found by Fay et al. (2000). *Hesperocallis* was moderately supported as sister to *Agave* (74% BS), but strongly supported (100% BS) as being a member of a clade that included Agavaceae (*sensu* APG 1998), Anthericaceae, Behniaceae, and Herreriaceae. Together, these taxa formed a sister group to Anemarrhenaceae (89% BS). Indels (not coded in the matrix) also supported the relationship of *Hesperocallis* with *Agave*.

The data presented here provide clear evidence that *Hesperocallis* is related to Agavaceae rather than members of Alliaceae or Hemerocallidaceae. In terms of APG (1998), *Hesperocallis* is embedded in a clade that includes Agavaceae, Anemarrhenaceae (monogeneric), Anthericaceae, and Behniaceae (monogeneric). In contrast to APG (1998), the APG II (2003) classification expands Agavaceae to include Anemarrhenaceae, Anthericaceae, Behniaceae and Herreriaceae. Based on our results (Fig. 1), we recommend that Hesperocallidaceae be treated as a synonym of Agavaceae (*sensu* APG II 2003) in the higher Asparagales.

However, making Hesperocallidaceae synonymous with Agavaceae is complicated by the fact that APG II has a "bracketed system" for the classification of the higher Asparagales. This system allows for the option of smaller bracketed families (such as the expanded Agavaceae) to be recognized within larger families of the APG II system. Specifically, APG II (2003) further simplified the higher Asparagales into two newly circumscribed large families, Asparagaceae s.l. and Alliacaeae s.l. In this sense, Agavaceae (with *Hesperocallis*) would simply be within Asparagaceae s.l., along with Aphyllanthaceae, Asparagaceae, Hyacinthaceae, Laxmanniaceae, Ruscaceae and Themidaceae.

Future studies will resolve the phylogenetic relationship of *Hesperocallis* to other taxa such as *Camassia, Chlorogalum, Hosta, Hesperaloe, Hesperoyucca,* and the other genera of the *Agave-Yucca* clade (Bogler and Pires unpublished data).

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